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Search for Water-Soluble, Nontoxic Fullerene Derivatives to Inhibit HIV-I Protease Activity

Christopher J. Leichliter

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Until recently it was thought that pure carbon existed in only two forms or allotropes, named graphite and diamond. The difference between the two allotropes can be found in the arrangement of carbon atoms to each other. Graphite is a soft, black solid that conducts electricity and can be used as a lubricant. When graphite is subjected to extreme pressure underground for many years, the graphite becomes diamond, the hardest natural substance known. Graphite is composed of sheets of six-membered rings that are *sp2*-hybridized, allowing for electrons to flow along the sheets and for the sheets to slide past each other. Diamond is tetrahedrally arranged in a three-dimensional crystal network. The structures of graphite and diamond help to explain the characteristics of the allotropes.[1] In 1985 chemists at Rice University discovered another stable allotrope of carbon which possessed the molecular formula C_{60}. This new form of carbon subsequently became known as the fullerene.

The discovery of fullerenes, otherwise known as buckyballs, has produced an extensive search into the possible applications of this unique form of carbon. Spherically shaped, this hollow cluster of carbon atoms has special properties that include heat resistance and electrical conductivity, making it a possible candidate for semiconductors, micro-filters, and high temperature lubricants.[2] Another property of this molecule is its hydrophobic nature which can be exploited for its interactions with the hydrophobic cleft found in the active site of some enzymes. The enzyme HIV-protease possesses an active site that resembles the size and shape of the C_{60} fullerene.[3] Studies

have indicated that the active site is a hollow hydrophobic cylinder with Aspartate 25 and Aspartate 125 acting as polar catalytic groups.[4] If the fullerene were to become lodged in the active site of the enzyme, the protease would become inactivated, thus hindering the spread of the virus. This paper discusses the research conducted to discover and produce a water-soluble, nontoxic fullerene derivative to inhibit HIV-1 protease activity.

Using computer molecular modeling, a team of researchers at the University of California San Francisco[5] made models of C_{60} and the protease enzyme. After minimizing the models, the fullerene was fitted into the active site of the enzyme using a program called DOCK3. This program aligns the fullerene with the active site by optimizing the ligandreceptor interactions of the van der Waals contacts and complementary electrostatics.[6] The lowest energy conformer was selected for subsequent energy studies. An estimation of the energy associated with this interaction can be approximated by calculating the difference in solvent-exposed surface area between the open active site and the C_{60} / enzyme complex. Once bound, the fullerene effectively removes 298 $Å^2$ of surface area from solvent contact. This calculation corresponds to a free energy gain of approximately 8-12 kcal/mol for the bound complex.[7] A few of the other factors involved in the energetics, including the aspartate interactions with the C_{60} surface and the conformational energy of the bound complex, were left out of the estimation for simplicity. The dissociation constant K_i can be estimated using the relationship $\Delta G_{bind} = -RT\ln K_i$, producing a constant of about 10^{-6} to 10^{-9} M. Other HIV protease inhibitors, as Friedman notes, lie in the subnanomolar range (peptide based) and high nanomolar range of concentrations (nonpeptide based). Lower dissociation constants indicate tighter binding of the enzyme-inhibitor complex, which is necessary for removal of active enzyme.

The problem surrounding the use of C_{60} centers on its hydrophobicity, making it insoluble in a water environment. Solubility can be achieved, however, if the fullerene possesses polar substituents that can interact with the

water solvent. Researchers have constructed several derivatives, with varying potency, to test against HIV activity.[8] The first attempt to create a water-soluble, thermally stable fullerene derivative in the range of physiological pH produced bis (phenethylamino-succinate) C_{60}.[9] This compound was produced via cycloaddition to the fullerene with a diphenyldiazomethane derivative. After creating the first anti-HIV fullerene derivative, Friedman calculated the K_i to be 5.3 μM for the competitive inhibitor. Emory University School of Medicine's Schinazi tested this derivative with HIV infected human lymphocytes to obtain an EC_{50} of ~ 6 μM.[10] (EC_{50} indicates the effective enzyme concentration with 50% maximum activity.)

Many people infected with HIV can attest that the treatment of the virus can be as painful as the actual infection. The adverse reactions associated with the most effective drugs can be related to the cytotoxicity of the drug on uninfected cells. Using peripheral blood mononuclear cells, H9 cells, Vero cells, and CEM cells, Schinazi found the fullerene derivative to be nontoxic up to a concentration of 100 μM. This indicates that most of the first generation fullerene derivatives are comparatively safe for regular functioning cells.

Currently the search for HIV inhibition with fullerenes involves the discovery of novel substituents to attach to the fullerene. One group sought to improve the substituent by using a pentapeptide known to display strong human monocyte chemotaxis.[11] This peptide sequence induces monocyte migration, which would concentrate the fullerene derivative around the cells that are attacked by the HIV virus. Preliminary results indicate similar anti-viral activity compared to other fullerene derivatives. These peptide-fullerenes will probably dominate the newly synthesized derivatives in the search for better inhibitors.

While the results using the current "first generation" fullerene derivatives are promising, they still lack the potency of current anti-HIV technology.[12,13] There are problems with protease inhibition that make it difficult to find any effective inhibitor for the HIV protease enzyme. As Schinazi explains, the

manufactured drug must be resistant to enzyme degradation and possess a low protein binding affinity. It must also be able to penetrate the virus membrane or be at sufficient concentration near the cell membrane prior to virus budding. These obstacles make the search for potent inhibitors very difficult.

The use of C_{60} to produce an HIV inhibitor is a novel approach in the search for new uses of fullerenes. With the Nobel Prize for Chemistry recently awarded to the discoverers of fullerenes, it is apparent that this new form of carbon has become an interesting topic for research throughout the sciences.[14] Although the current biological applications of C_{60} are limited, this field promises to produce an exciting aray of new discoveries.

Notes

[1] Chang, R. *Chemistry*, Fifth Edition, 1994, 53, 447.

[2] http://www.ornl.gov/ORNLReview/rev26-2/text/rndmain1.html and http://www.chem.sunysb.edu/msl/fullerene.html

[3] A graphical representation of HIV protease can be seen in the reference section of this document. Obtained from http://florey.biosci.uq.oz.au/hiv/lecture/cpk_dimer.html

[4] Erickson, J. et al. *Science* 1993, Vol. 249, 527.

[5] Friedman, S.H.; DeCamp, D.L.; Sijbesma, R.P.; Srdanov, G.; Wudl, F.; and Kenyon, G.L. "Inhibition of the HIV-1 Protease by Fullerene Derivatives: Model Building Studies and Experimental Verification." *Journal of the American Chemical Society* 1993, Vol. 115, 6506-6509.

[6] Meng, E.C.; Shoichet, B.K.; Kuntz, I.D. *Journal of Computational Chemistry* 1992, Vol. 13, 505-524.

[7] The free energy gain associated with desolvation has been estimated to be 69.2 cal / (mol * Å2). Friedman cited the following source for this estimation: Tunon, I.; Silla, E.; Pascual-Ahuir, J.L. *Protein Engineering* 1992, Vol. 5, 715-6.

The energy loss due to the change of translational/rotational entropy is on the order of 7 to 11 kcal/mol. Friedman credited this estimation to: Novotny, J.; Bruccoleri, R.E.; Saul, F.A. *Biochemistry* 1989, Vol. 28, 4735-49.

$\Delta G_{bind} = \Delta H - T\Delta S$
ΔG_{bind}[7] = 69.2 cal / (mol * Å^2) * 298 Å^2 - 7 to 11 kcal/mol = 8 to 12 kcal/mol

[8] Schuster, D.I.; Wilson, S.R.; Schinazi, R.F. "Anti-Human Immunodeficiency Virus Activity and Cytotoxicity of Derivatized Buckminsterfullerenes." *Bioorganic & Medicinal Chemistry Letters* 1996, Vol. 6, No 11, 1253-6.

[9] Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J.A.; Wilkins, C.; Friedman, S.H.; DeCamp, D.L.; and Kenyon, G.L. "Synthesis of a Fullerene Derivative for the Inhibition of HIV Enzymes." *Journal of the American Chemical Society* 1993, Vol. 115, 6510-2.

[10] Schinazi, R.F.; Sijbesma, R.; Srdanov, G.; Hill, C.L.; Wudl, F. "Synthesis and Virucidal Activity of a Water-Soluble, Configurationally Stable, Derivatized C_{60} Fullerene." *Antimicrobial Agents and Chemotherapy* 1993, 1707-10.

[11] Toniolo, C.; Bianco, A.; Maggini, M.; Scorrano, G.; Prato, M.; Marastoni, M.; Tomatis, R.; Spisani, S.; Palu, G.; Blair, E. "A Bioactive Fullerene Peptide." *Journal of Medicinal Chemistry* 1994, Vol 37, 4558-4562.

[12] Schuster, D.; Wilson S.; Schinazi, R. "Anti-human Immunodeficiency Virus Activity and Cytotoxicity of Derivatized Buckminsterfullerenes." *Bioorganic & Medicinal Chemistry Letters* 1996, Vol 6, No. 11, 1253-1256.

[13] A fullerene derivative with an anti-HIV activity of EC_{50} of 0.9 μM has recently been produced by the group of footnote 11.

[14] For more information regarding Curl, Kroto or Smalley, the 1996 Nobel Prize winners in chemistry: www.nobel.se/announcement96/chemistry96.html